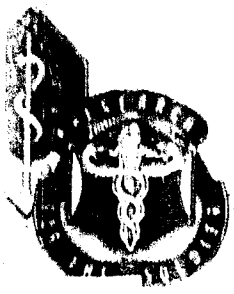

Institute Report No. 270

Circulatory and Hematological Effects of Liquid Propellant 1846 Following Oral Administration to Rats

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and
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DIVISION OF TOXICOLOGY

June 1988

Toxicology Series: 189

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Circulatory and hematological effects of liquid propellant 1846 following oral administration to cats (Toxicology Series 189)--Orner et al.

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In conducting the research described in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council.

This material has been reviewed by Letterman Army Institute of Research and there is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. *(AR 360-5)*

Edwin S. Beatrice 10 June 87

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UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: UNCLASSIFIED	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION / AVAILABILITY OF REPORT: Unlimited
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S): Institute Report No. 270	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Division of Toxicology; 6b. OFFICE SYMBOL (If applicable): SGRD-UL-TO	7a. NAME OF MONITORING ORGANIZATION: United States Army Biomedical Research and Development Laboratory
6c. ADDRESS (City, State, and ZIP Code): Letterman Army Institute of Research Presidio of San Francisco, CA 94129-6800	7b. ADDRESS (City, State, and ZIP Code): Fort Detrick, MD 21701-5010
8a. NAME OF FUNDING / SPONSORING ORGANIZATION: United States Army Medical Research and Development Command; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: Project Order 86PP6823 Toxicity Study of Liquid Gun Propellant, LP1846
8c. ADDRESS (City, State, and ZIP Code): Fort Detrick, MD 21701-5012	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. / PROJECT NO. / TASK NO. / WORK UNIT ACCESSION NO.

11. TITLE (Include Security Classification)
Circulatory and Hematological Effects of Liquid Propellant 1846 Following Oral Administration to Rats

12. PERSONAL AUTHOR(S)
Gayle A. Orner, SGT USA, Danley F. Brown, DAC, and Don W. Korte, Jr., MAJ, MS

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final	FROM 10 Jun TO 100ct86	June 1988	47

16. SUPPLEMENTARY NOTATION
Toxicology Series 189

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
Heinz Bodies, Methemoglobin, Hypotension, Liquid Propellants, Hydroxylammonium Nitrate, HAN, Rats, Nitrates, LP1846

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Hydroxylammonium nitrate (HAN), a major component of the liquid propellant 1846 (LP1846), has been reported to produce methemoglobinemia, Heinz body formation, and hypotension in rats. This study was conducted to define the relative sensitivity and reversibility of the methemoglobinemia, hypotension, and Heinz body formation following oral administration of LP1846 to male rats. The left carotid artery of each Sprague-Dawley rat was surgically implanted with customized polyurethane catheters. After a 3- to 5-day recovery period, the animals were assigned to one of three groups and were administered a single dose of either sterile water (control) or LP1846 (equivalent to 50 or 100 mg/kg of HAN) by oral gavage. Blood pressure was monitored before dosing and for 60 minutes following dosing. Blood samples for determining the presence of methemoglobin and identifying Heinz bodies were obtained at -1, 5, 15, 30, 60, 120, 180, 240, 300 and 360 minutes after dosing, and then at (Cont.)

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION	
☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS	Unclassified	
22a. NAME OF RESPONSIBLE INDIVIDUAL: Edwin S. Beatrice	22b. TELEPHONE (Include Area Code): (415) 561-3600	22c. OFFICE SYMBOL: SGRD-UL-Z

DD Form 1473, JUN 86 — Previous editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

19. 24-hour intervals until methemoglobin values returned to normal. LP1846 had no effect on mean arterial pressure at a dose of 50 mg(HAN)/kg, but a dose of 100 mg(HAN)/kg produced a significant decrease of 14% at five minutes. LP1846 produced a dose-related increase of 5.2% and 10.6% in methemoglobin values for the 50 and 100 mg(HAN)/kg groups, respectively. The half-times for methemoglobin reduction were 113.0 hrs for the 50 mg(HAN)/kg and 93.2 hrs for the 100 mg(HAN)/kg groups. The times to the maximum methemoglobin concentration were 1.27 hrs for the 50 mg(HAN)/kg and 2.63 hrs for the 100 mg(HAN)/kg groups. Heinz bodies were present in all treated animals; the time of first observance being significantly shorter in the high-dose group. These data suggest that the presence of elevated methemoglobin levels and/or Heinz bodies would be useful indices of occupational exposure to LP1846.

ABSTRACT

Hydroxylammonium nitrate (HAN), a major component of the liquid propellant 1846 (LP1846), has been reported to produce methemoglobinemia, Heinz body formation, and hypotension in rats. This study was conducted to define the relative sensitivity and reversibility of the methemoglobinemia, hypotension, and Heinz body formation following oral administration of LP1846 to male rats. The left carotid artery of each Sprague-Dawley rat was surgically implanted with customized polyurethane catheters. After a 3- to 5-day recovery period, the animals were assigned to one of three groups and were administered a single dose of either sterile water (control) or LP1846 (equivalent to 50 or 100 mg/kg of HAN) by oral gavage. Blood pressure was monitored before dosing and for 60 minutes following dosing. Blood samples for determining the presence of methemoglobin and identifying Heinz bodies were obtained at -1, 5, 15, 30, 60, 120, 180, 240, 300, and 360 minutes after dosing, and then at 24-hour intervals until methemoglobin values returned to normal. LP1846 had no effect on mean arterial pressure at a dose of 50 mg(HAN)/kg, but a dose of 100 mg(HAN)/kg produced a significant decrease of 14% at five minutes. LP1846 produced a dose-related increase of 5.2% and 10.6% in methemoglobin values for the 50 and 100 mg(HAN)/kg groups, respectively. The half-times for methemoglobin reduction were 113.0 hrs for the 50 mg(HAN)/kg and 93.2 hrs for the 100 mg(HAN)/kg groups. The times to the maximum methemoglobin concentration were 1.27 hrs for the 50 mg(HAN)/kg and 2.63 hrs for the 100 mg(HAN)/kg groups. Heinz bodies were present in all treated animals; the time of first observance being significantly shorter in the high-dose group. These data suggest that the presence of elevated methemoglobin levels and/or Heinz bodies would be useful indices of occupational exposure to LP1846.

Key words: Heinz Bodies, Methemoglobinemia, Hypotension, Liquid Propellants, Hydroxylammonium nitrate, HAN, Rats, Nitrates, LP1846.

PREFACE

TYPE REPORT: Rodent circulatory and hematological toxicity study

TESTING FACILITY:
US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:
US Army Medical Research and Development Command
US Army Biomedical Research and Development Laboratory
Fort Detrick, MD 21701-5010
Project Officer: Robert Finch, PhD

PROJECT/APC: USABRDL Reimbursable Service Order 8620/F844, Project Order 86PP6823 (BASIC), TL1R

GLP STUDY NUMBER: 86004

STUDY DIRECTOR: MAJ Don W. Korte, Jr., PhD, MS

PRINCIPAL INVESTIGATORS: SGT Gayle A. Orner, BS
Danley F. Brown, PhD

REPORT AND DATA MANAGEMENT : A copy of the final report, study protocol, retired SOPs, raw data, analytical stability and purity data for the test compound, and an aliquot of the test compound will be retained in the LAIR Archives

TEST SUBSTANCE: LP1846

INCLUSIVE STUDY DATES: 10 June 1986-10 October 1986

OBJECTIVE: The objective of this study was to define the relative sensitivity and reversibility of the methemoglobinemia, hypotension, and Heinz body formation following administration of a single oral dose of LP1846 to male rats.

ACKNOWLEDGMENTS

The Division of Military Trauma Research (MTR) provided the use of a co-oximeter, pressure transducers, and Gould recorders. SGT Carol Bossone and SSG Sharon Velez provided expertise and were especially cooperative in assisting us with the use of the MTR equipment, and LTC John R. Hess from the Division of Blood Research identified and photographed the Heinz bodies. LTC C. Pamplin and Dr. Ginny Gildengorin assisted in the statistical analysis of the data. SGT Gregory Rothammer, SP4 Theresa L. Polk, SP4 Scott L. Schwebe, and Richard A. Spieler provided animal care and technical assistance. SSG James D. Justus and SP4 Joel B. Seewald provided research assistance. Colleen S. Kamiyama provided secretarial assistance.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 86004 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte Jr. 10 MAY 88

DON W. KORTE JR., PhD / DATE
MAJ, MS
Study Director

Gayle A. Orner 10 May 88

GAYLE A. ORNER, B.S. / DATE
SGT, USA
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Stanley F. Brown 4 MAY 88

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DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF

SGRD-ULZ-QA 10 May 1988

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance Statement

1. This is to certify that the protocol for GLP Study 86004 was reviewed on 6 June 1986.

2. The institute report entitled "Circulatory and Hematological Effects of Liquid Propellant 1846 Following Oral Administration to Rats," Toxicology Series 189, was audited on 26 August 1987.

Carolyn M. Lewis
CAROLYN M. LEWIS
Chief, Quality Assurance

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APPENDICES

Circulatory and Hematological Effects of LP1846 Administration to Rats--Orner *et al*

INTRODUCTION

Liquid propellants are being evaluated by the Armed Forces because they offer several important advantages over conventional solid propellants. They are less expensive to produce and transport, are less vulnerable to secondary ignition, have increased ability to sustain operations, are easier to store in combat vehicles, and can be demilitarized simply and safely (1). Since there is considerable potential for human contact with the liquid propellants during manufacture, transportation, and use, their health effects are of interest to the Army. Previous studies on the monopropellant hydroxylammonium nitrate (HAN), the pharmacologically active component of the liquid propellant 1846 (LP1846), demonstrated that HAN induced hypotension, methemoglobinemia, and Heinz body formation in dogs and rats (2,3). The Division of Toxicology, Letterman Army Institute of Research (LAIR), was tasked by the United States Army Biomedical Research and Development Laboratory (USABRDL) to determine the relative sensitivity and reversibility of these indices following LP1846 administration.

Objective

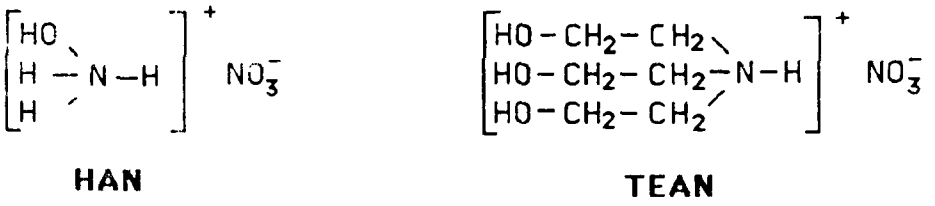
The objective of this study was to define the relative sensitivity and reversibility of the methemoglobinemia, hypotension, and Heinz body formation following administration of a single oral dose of LP1846 to male rats.

MATERIALS AND METHODS

This study was conducted in compliance with the Good Laboratory Practices (GLP) regulations promulgated by the Environmental Protection Agency (4). LAIR is an AAALAC-approved animal care facility (American Association for Accreditation of Laboratory Animal Care, 208A North Cedar Road, New Lenox, IL). All research conformed to standards established in the Guide for the Care and Use of Laboratory Animals and with other applicable federal regulations.

Test Substance

LP1846 (Lot #50-4) was obtained from the United States Ballistic Research Laboratory (Aberdeen Proving Ground, MD). LP1846 contains 61.5% hydroxylammonium nitrate (HAN), 18.4% triethanolammonium nitrate (TEAN), and 20.1% water (Fig. 1).



HAN **TEAN**

Figure 1. Components of LP1846

Vehicle

Since the water component acts as a diluent and maintains the mixture in a liquid state, sterile water for injection was selected as the vehicle for formulating the dosing solutions. The dose groups (50 and 100 mg(HAN)/kg) were calculated based on the reported concentration of the HAN component in the LP1846. The final concentrations based on in-house analyses were 83.3 and 166.7 mg(LP1846)/kg. Other test substance information is presented in Appendix A.

Animal Data

Male Sprague-Dawley rats were used for the study (Bantin & Kingman Inc., Fremont, CA). The animal weights at dosing ranged from 227 to 380 grams. The animals were housed individually in stainless steel, wire meshed cages with automatically flushing dumptanks. The diet consisted of certified Purina Rodent Chow® #5022 (Ralston Purina Company, St. Louis, MO); purified water from a Technic Series 300 Reverse Osmosis Unit (Seattle, WA) was provided from a central line. The animal room temperature was maintained at 22°-25° C with a relative humidity range of 36%-53%. The photoperiod was 12 hours of light per day. Additional animal data can be found in Appendix B.

Catheterization Surgery

The rats were anesthetized with 0.5 ml/kg Innovar-Vet® (Janessen Pharmaceutical Inc., New Brunswick, NJ). All surgeries were performed using aseptic procedures. Catheterizations were performed using the procedures developed by Brown and coworkers (5-8). An incision (approx. 2 cm) was made on the ventral surface of the neck, and the muscles were separated to expose the left carotid artery. A small cut was made in the artery, and a polyurethane catheter (inner diameter, 0.69 mm; outer diameter, 1.02 mm) was inserted approximately 2.5 cm towards the heart and sutured in place. The catheter was heparinized (9:1 glycerol:heparin [1000 units/ml] solution), externalized on the dorsal surface of the neck, and held in place with a Velcro® patch (Smalley and Bates Inc., Nutley, NJ).

Allocation/Acceptance Criteria

Following the 3- to 5-day recovery period, animals were assigned sequentially to a test group as follows: 0, 50, 100, 0, 50, 100, 0... until a minimum of 8 test animals had been assigned to each group. Since the study objective was to describe the relative sensitivity and reversibility of LF1846's major pharmacological effects, acceptance criteria were that each experiment should run a minimum of 24 hours and sufficient blood samples should be obtained during this time to define the half-time of methemoglobin reduction. These criteria were achieved even though there were some deaths attributable to acute cardiovascular collapse and even though other animals had to be removed from the study due to loss of catheter patency or displacement. These acceptance criteria were adopted with the understanding that the acute hemodynamic effects might be understated because those

animals that died in acute cardiovascular collapse were eliminated from the study.

Test Procedures

Animals were removed from their cages 3 to 5 days following catheter implantation and acclimated to the container in which the procedures were performed. Catheter patency was confirmed. These catheters were then filled with a solution of heparinized saline (9:1 saline:heparin [1000 units/ml]) and attached to a Stathem DB transducer (Stathem Laboratories, Hato Rey, Puerto Rico). The transducer signal was monitored via a Gould 2200 polygraph (Cleveland, OH) and electrically dampened to obtain mean arterial pressure (9). LP1846 and vehicle were administered by oral gavage. Blood pressure was recorded from five minutes before dosing to 60 minutes after LP1846 administration. Time periods selected for analysis of mean arterial pressure were at -1 (baseline), 5, 15, 30, and 60 minutes after dosing. Heparinized blood samples (0.25 ml) for determining methemoglobin and identifying Heinz bodies (10) were collected at -1 (baseline) and 5, 15, 30, 60, 120, 180, 240, 300 and 360 minutes, and then at 24-hour intervals after dosing until methemoglobin values had returned to normal or an animal was removed from study because of catheter patency failure after the 24-hour minimal acceptance period. Blood samples were refrigerated until measurements were made. Methemoglobin values were determined using a Model 282 Co-oximeter (Instrumentation Laboratories, Lexington, MA) (11). Fresh blood samples were stained with crystal violet to determine the presence of Heinz bodies (12).

Duration of Study

Appendix C is a complete list of historical events.

Statistical Analysis

Randomness of allocation procedures was confirmed for the baseline blood pressure and methemoglobin values by one-way analysis of variance (ANOVA). Since there were no significant differences in the baseline mean arterial pressures, the dose groups were compared using a two-way analysis of covariance with time as a repeated measure and the baseline values as a covariate. The multivariate t-method was then used to make the planned comparisons. Then a one-way analysis of variance was performed to test for differences between baseline and the 5, 15, 30, and 60 min. time points for each group. The change from baseline at each time point was compared by one-way ANOVA and, if a

significant F value was obtained, Dunnett's t-test was performed (13, 14). These statistical analyses were performed with the BMDP statistical software package (15) on the Data General MV/8000 computer. The methemoglobin data were analyzed by nonlinear regression using a modified Marquardt algorithm for non-linear least squares regression (16). The data were fitted to a one-compartmental open model, and toxicokinetic indices (half-time of methemoglobin reduction, peak concentration, time-to-peak concentration, and area under the time-concentration curve) were derived. A student's t-test was used to compare the kinetic indices for the two test groups. The presence of Heinz bodies was evaluated qualitatively. Time of appearance of Heinz bodies in the two test groups was compared using the student's t-test. All tests were performed at the .05 level of significance.

Changes/Deviations from Procedures

Originally, blood pressure tracings were to be taken before dosing, at 5, 15, 30, and 60 minutes after dosing, and then at one-hour intervals for six hours. Preliminary studies indicated that the significant blood pressure changes occurred within the first hour. The times of recording were changed to enable closer monitoring of the first hour, particularly the first 15 minutes. The timetable was changed to monitor the blood pressure continuously from one minute before to 15 minutes following dosing, and then at five-minute intervals for the first hour. No pressure tracings were taken after the first hour.

Due to the relatively long half-time for methemoglobin reduction following LP1846 administration in rats, there was some difficulty in maintaining catheter patency during the period required for the methemoglobin values to return to baseline. Starting on 26 Aug 1986, we used a blood sample from the tail vein of the animal if the catheter was no longer patent.

The protocol indicates that the animals would be allowed to recover for three days following catheterization. This was changed to a recovery period of three to five days. This allowed the catheterization of more than two rats in a day which provided backups on the day of dosing in case the catheters of the animals scheduled to be dosed were not patent. Extra animals could be dosed the following day, rather than being eliminated from the study. The recovery of rats from catheterization surgery is complete after three days (6). An extra day or two of recovery should not have made a difference in the study results.

Two power outages occurred during the course of the study. On 25 June and on 19 July the animals were without water for approximately four hours. The room humidity and temperatures were not stable during these times. The animals were also without water for a period between the p.m. observations on 5 Sept. and the a.m. observations on 6 Sept. No signs of dehydration were noted after these occasions.

Raw Data and Final Report Storage

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

RESULTS

Blood Pressure

The mean and standard error of the mean (SEM) for the blood pressure measurements (at the prescribed time periods) are given in Table I for each group. Only the 100 mg (HAN)/kg dose of LP1846 produced a significant decrease in mean arterial pressure (MAP). The 17.3 mm Hg decrease in MAP at 5 minutes after oral administration was significantly greater than the decrease in MAP observed in the control or 50 mg (HAN)/kg group. Appendix D is a listing of the mean arterial pressures of the test animals.

Methemoglobinemia

LP1846 produced a prolonged dose-related methemoglobinemia in the rat following oral administration. Methemoglobin kinetic values are given in Table II. The half-time for methemoglobin reduction (T-1/2 Red) was approximately 4 days for both doses, and the time to maximum methemoglobin concentrations (T-max) was 1.27 hrs and 2.63 hrs for the 50 and 100 mg(HAN)/kg dose groups, respectively. There were no significant differences in these two variables between the two groups. The area under the curve (AUC) described by the time vs % methemoglobin plot was significantly larger in the 100 mg(HAN)/kg than in the 50 mg(HAN)/kg dose group as was the maximum methemoglobin concentration (C-max). Appendix E contains the concentration versus time curves for the methemoglobinemia observed in the 50 and 100 mg(HAN)/kg dose groups, respectively. The half-time for methemoglobin reduction is listed under the T-1/2 column of the parameters as Term 2. Methemoglobin values averaged between 1.1% and 1.3% in the

control group throughout the observation period which extended 6 days or longer for some experiments. Appendix F is a listing of the methemoglobin values for all animals and Appendix G shows the methemoglobin kinetics.

Heinz Body Formation

No Heinz bodies were observed in blood samples from control group animals at any time period. Heinz bodies were observed in the blood samples of all treated rats. Figure 2 is a graphical representation of the time of appearance of Heinz bodies in the 50 and 100 mg(HAN)/kg groups. There was a significant decrease in onset of time of appearance of Heinz bodies from the 50 to 100 mg(HAN)/kg group. The disappearance of Heinz bodies from the circulation was difficult to monitor because of periodic losses of catheter patency and animals from the study as the observation period continued. However, the data suggest that the time of disappearance of the Heinz bodies parallels the reduction in methemoglobin. At the 100 mg(HAN)/kg dose essentially every erythrocyte had Heinz bodies. The percentage of erythrocytes with Heinz bodies was much more variable in the 50 mg(HAN)/kg group. Some animals had blood samples essentially the same as those in the 100 mg(HAN)/kg group while other animals had very few Heinz bodies. Appendix H is a complete listing of the times for first appearance of the Heinz bodies.

TABLE I

The Effect of Orally Administered LP1846 on Mean Arterial Pressure (mm Hg) in Conscious Rats[a]

Group	Time (min) After Dosing				
	-1[b]	5	15	30	60
Control	118.4	118.4	123.0	120.9	118.8
(n=8)[c]	±3.5	±3.5	±4.7	±3.2	±9.2
50 mg(HAN)/kg	120.1	117.4	121.0	115.6	122.9
(n=8)	±5.7	±4.0	±5.1	±8.5	±6.9
100 mg(HAN)/kg	123.6	106.3[d,e]	119.4	123.4	122.4
(n=8)	±4.4	±7.3	±7.3	±6.8	±8.4

a. Values are mean ± standard error of the mean.

b. Measurements were obtained one minute before dosing and were considered baseline observations.

c. Animal #393 was not included in the statistical analysis due to several missing data points.

d. Significantly different from baseline (-1 min) when compared using one-way ANOVA and Multivariate t-method, $p \leq 0.05$.

e. Change from baseline for the 100 mg(HAN)/kg dose group was significantly different from the change from baseline for the vehicle control when compared using one-way ANOVA and Dunnett's t-test, $p \leq 0.05$.

TABLE II

Methemoglobin Kinetics Following Oral Administration of LP1846 to the Conscious Rat[a,b]

Group[c]	T-1/2 Red (hrs)	AUC (% hrs)	T-max (hrs)	C-max (%)
50 mg(HAN)/kg (n=8)	113.1 ±9.4	848.3 ±87.3	1.27 ±0.15	5.2 ±0.5
100 mg(HAN)/kg (n=8)	93.2 ±12.4	1358.0[d] ±100.9	2.63 ±1.22	10.6[d] ±1.1

a. Abbreviations:
- T-1/2 Red: half-time for methemoglobin reduction
- AUC: area under the curve described by the methemoglobin vs time plot
- T-Max: time to maximum methemoglobin concentration
- C-max: maximum methemoglobin concentration

b. Values are mean ± standard error of the mean.
c. Control Methemoglobin values did not change from baseline. No kinetic analysis was attempted.
d. Significantly different from 50 mg(HAN)/kg: Student's t-test, $p \leq 0.05$.

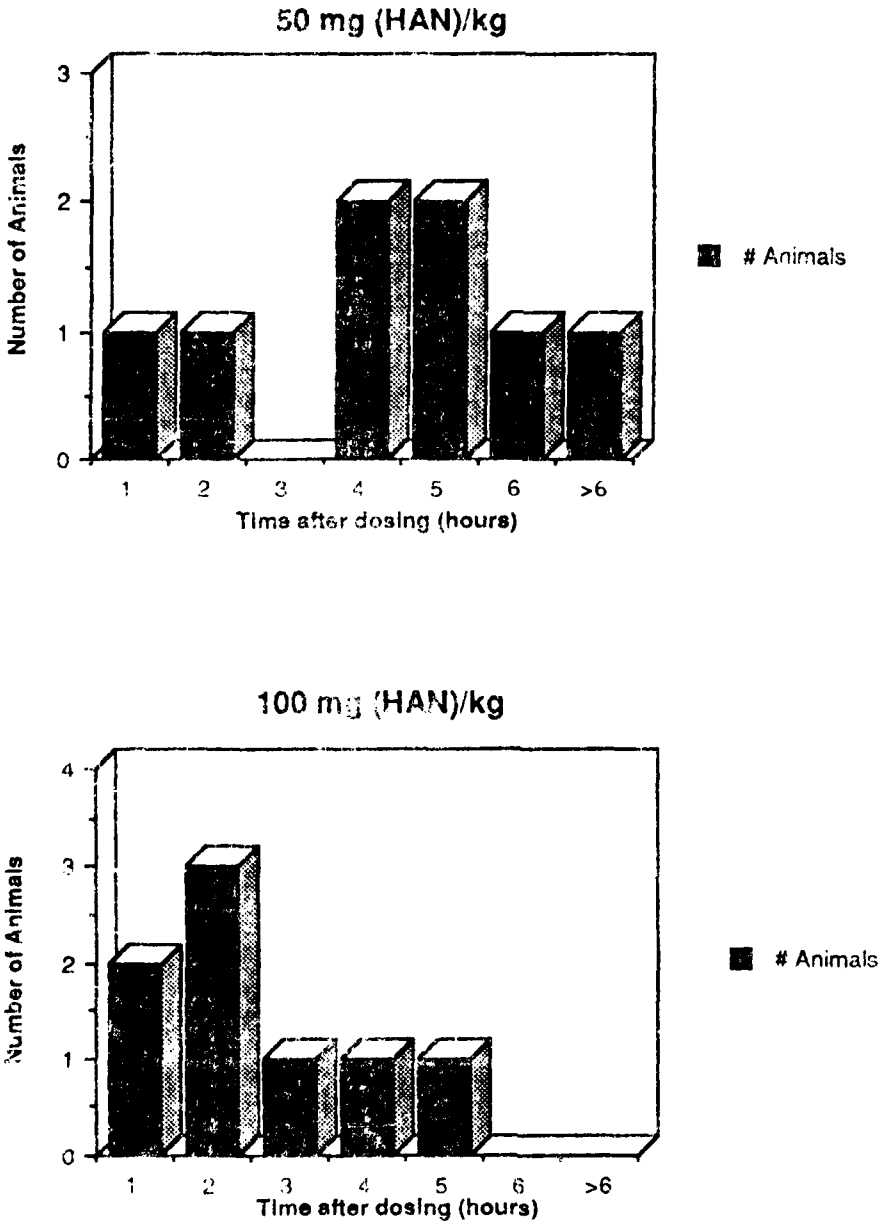


Figure 2. HEINZ BODY FORMATION
(time at first observed)

DISCUSSION

The objective of this study was to describe the relative sensitivity and the reversibility of LP1846 on certain hemodynamic and hematological variables. It has previously been established that liquid propellants containing HAN produce a profound systemic hypotension and methemoglobinemia, plus other blood dyscrasias, in the dog and rat (2). Preliminary studies in this laboratory confirmed the acute toxicity of the HAN-containing liquid propellant, LP1846, in the rat. A single orally administered dose of LP1846, 200 mg(HAN)/kg, equivalent to approximately one-half the median lethal dose, produced at 3 hours a 50% decrease in MAP, which had returned to baseline values by 6 hours. In the same animal, LP1846 produced a 46% methemoglobinemia 15 minutes after administration. Sixty-six hours after dosing, the methemoglobin concentration in this animal still exceeded 18%. On the basis of these data, two doses of LP1846 were selected to determine the relative sensitivity and reversibility of LP1846 effects on blood pressure, methemoglobin concentrations, and Heinz body formation. The two doses of LP1846 were 50 and 100 mg(HAN)/kg. Doses were based on the concentration of HAN in the LP1846 mixture as previous studies had indicated that HAN was the active component for the hemodynamic and hematological changes observed following liquid propellant administration (2,17).

Our study established that 100 mg(HAN)/kg of LP1846 administered orally produced a significant yet transient hypotension in conscious rats. As described in the acceptance criteria section, the magnitude of the hypotensive response may have been understated since the requirement for a successful experiment was that the animal remain "on study" for at least 24 hours. However, this requirement was necessary to ensure sufficient successful experiments for determining the time course for the methemoglobinemia and Heinz body formation. The lack of a hypotensive effect at the 50 mg(HAN)/kg dose level suggests that the threshold for this response is between 50 and 100 mg(HAN)/kg.

LP1846 produced a dose-related methemoglobinemia as indicated by peak concentration and area-under-the-curve measurements. The relationship between the peak methemoglobin concentration and dose of LP1846 was essentially linear with methemoglobin values of 5.2% and 10.6% for the 50 and 100 mg(HAN)/kg doses, respectively. Thus, the methemoglobin concentration appears to be a sensitive indicator of LP1846 (e.g., HAN) in the blood. There were no significant differences in the half-times for methemoglobin reduction at the two dose levels. These data

varied more than the peak concentration data because there were fewer data points from the later sampling periods which, if present, would have provided a more accurate description of the half-time for methemoglobin reduction. The relatively long half-time (approximately 4 days) for methemoglobin reduction following LP1846 administration reflects either a depression of methemoglobin reductase activity by HAN or a relatively long elimination half-time for HAN or its active methemoglobin-forming metabolites. The prolonged sodium nitrate-induced methemoglobinemia in mice has been attributed to methemoglobin reductase inhibition (18). However, this may not be the case in the rat. The rat has a relatively fast-acting methemoglobin reductase and there is a good correlation between methemoglobin time profiles and elimination profiles for methemoglobin-forming drugs such as dimethylaminophenol and p-aminopropiophenone (19). Therefore we may speculate that the long half-time for methemoglobin reduction is attributable to a long half-time of elimination for HAN. Studies of the metabolic fate of LP1846 (HAN) would answer these questions and provide much needed information for treating cases of accidental poisoning.

Formation of Heinz bodies in erythrocytes is characteristic of many oxidative compounds (20). Consequently, it has been proposed that Heinz body formation is directly associated with methemoglobin production (21). Although the consensus is that methemoglobin and Heinz body formation are not directly related, a definitive study has not been conducted. The LP1846 data do not help to resolve this old controversy. On one hand, the time courses for elimination of Heinz bodies and reduction of methemoglobin following administration of LP1846 are similar, although this comparison relies on a qualitative analysis for the presence of Heinz bodies. As discussed previously for the methemoglobin data, this time course of elimination may, in fact, reflect a long elimination half-time for LP1846 (HAN). Conversely, the data may suggest also that different mechanisms account for formation of methemoglobin and Heinz bodies because there is a dose-dependent decrease in time of appearance of Heinz bodies, whereas there is no significant difference in the time to maximum methemoglobin concentrations for the two doses of LP1846.

CONCLUSIONS

This study confirms earlier reports that HAN-containing liquid propellants produce hypotension, methemoglobinemia, and Heinz body formation. The data from this study indicate that all measured variables respond in a dose-related fashion

to LP1846 exposure. LP1846, or its HAN component, was more potent in producing methemoglobin and Heinz body formation than in producing hypotension. Consequently, either methemoglobin or Heinz body formation (or preferably both since their formation may not be mechanistically related) should be monitored periodically during occupational exposure. The hypotensive response was transient at the doses used in the study; larger doses produced longer, more prolonged periods of hypotension as is true with nitrate salts in general. The relatively long half-time of four days for methemoglobin reduction in the rat following LP1846 administration suggests that LP1846 may have a long half-time of elimination. Thus, one should monitor methemoglobin and Heinz body formation during periods of prolonged exposure to LP1846.

REFERENCES

1. Kirkpatrick DG (ed). Ballistic Research Laboratory studies liquid propellants. Army Research, Development, and Acquisition Magazine. May/June 1986: 25-26.

2. Metker LW, McCreesh AH, Thompson SA, Singer AW. Physiological and pharmacological effects of liquid gun propellants. Proceedings of the Eleventh Conference on Environmental Toxicology. AFAMRL-TR-80-125, 18-20 November 1980, Dayton, OH: 1981:232-240.

3. Metker LW, Thompson SA, Singer AW. Physiological and pharmacological effects following oral administration of monopropellants. Phase I, Special Study No. 75-51-0132-80. Aberdeen Proving Ground, MD: US Army Environmental Hygiene Agency, August 1978 - Sept. 1979.

4. Environmental Protection Agency, Toxic Substances Control Act, Good Laboratory Practice Standards (40 CFR 792). Final Rule, 29 November 1983 (48 FR 53922-53944).

5. Brown DF, Burr RE. Customized vascular catheters for rodents. Lab Animal Sci 1985; 35: 515-516.

6. Brown DF, Bellamy RF. The effect of WR 149024 on survival following hemorrhage in conscious rats. Toxicol Lett 1985; 26: 127-132.

7. Carotid cannulation in rats. LAIR Standard Operating Procedure OP-STX-97, Presidio of San Francisco, CA: Letterman Army Institute of Research, 15 May 1986.

8. Customizing polyurethane tubing to be used for vascular catheters. LAIR Standard Operating Procedure OP-STX-100, Presidio of San Francisco, CA: Letterman Army Institute of Research, 30 Apr 1986.

9. Recording blood pressure with a Gould recorder. LAIR Standard Operating Procedure OP-STX-96, Presidio of San Francisco, CA: Letterman Army Institute of Research, 30 Apr 1986.

10. Blood sample collection of arterial blood from the carotid artery of rats for methemoglobin analysis with the co-oximeter 282. LAIR Standard Operating Procedure OP-STX-98, Presidio of San Francisco, CA: Letterman Army Institute of Research, 15 May 1986.

11. Calibration and operation of the co-oximeter 282. LAIR Standard Operating Procedure OP-STX-99, Presidio of San Francisco, CA: Letterman Army Institute of Research, 30 Apr 1986.

12. Staining a blood sample for Heinz bodies. LAIR Standard Operating Procedure OP-STX-101, Presidio of San Francisco, CA: Letterman Army Institute of Research, 30 Apr 1986.

13. Milliken GA, Johnson DE. Analysis of messy data; Volume 1: Designed experiments. Belmont, CA: Lifetime Learning Publications, 1984.

14. Gad CG, Weil CS. Statistics for toxicologists. In: Hayes AW, ed. Principles and methods of toxicology. New York: Raven Press, 1982; 273-320.

15. BMDP statistical software. Berkeley, CA: University of California Press, 1983.

16. Pamplin CL. Personal Communication, Presidio of San Francisco, CA: Letterman Army Institute of Research, Division of Biophysical Research, 1987.

17. Snodgrass HL. Comparative inhalation toxicities of LGP 1776, LGP 1845, and HAN. CPIA Publication No. 436. Laurel, MD: Chemical Propulsion Information Agency (1985 JANNAF Safety and Environmental Protection Subcommittee Meeting), 1985:307-312.

18. Kruszyna R, Kruszyna H, Smith RP. Comparison of hydroxylamine, 4-dimethylaminophenol and nitrate protection against cyanide poisoning in mice. Arch Toxicol 1982; 49: 191-202.

19. Marrs TC, Bright JE. Kinetics of methemoglobin production (1). Kinetics of methemoglobinemia induced by the cyanide antidotes p-aminopropiophenone, p-hydroxyaminopropiophenone or p-dimethylaminophenol after intravenous administration. Hum Toxicol 1986; 5: 295-301.

20. Rentsch G. Genesis of Heinz bodies and methemoglobin formation. Biochem Pharmacol 1968; 17: 423-427.

21. Nagel RL, Ranney HM. Drug-induced oxidative denaturation of hemoglobin. Semin Hematol 10: 1973; 269-278.

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APPENDICES

CHEMICAL DATA

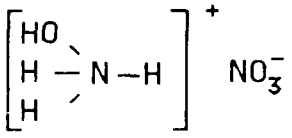
Chemical name: Liquid Gun Propellant 1846 (LP 1846)

Lot Number: 50-4

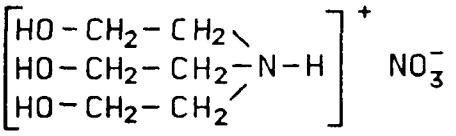
LPR Code: TP69

Propellant Components: hydroxylammonium nitrate (HAN)
triethanolammonium nitrate (TEAN)

Chemical Structures:



HAN



TEAN

Molecular Formula: HAN: $H_4N_2O_4$, TEAN: $C_6H_{15}N_2O_6$

Molecular Weight: HAN: 96.04, TEAN: 211.19

Analytical Data:

LP 1846 was analyzed by titration with a standardized alcoholic solution of potassium hydroxide using a procedure supplied by Charles Leveritt.[1] This yielded the sum of the HAN and TEAN components. Titration of the propellant after heating with benzaldehyde provided the concentration of HAN alone. Analysis of the compound in this laboratory gave the following composition:[2]

HAN	61.5%
TEAN	18.4%
Water	20.1%

Source: Charles S. Leveritt
Advanced Ballistic Concepts Branch
US Ballistics Research Laboratory
Interior Ballistic Division
Aberdeen Proving Ground, MD

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[1]Leveritt, CS. [Letter]. SUBJECT: Analysis of liquid propellants by titration (15 April 1986). Aberdeen Proving Ground, Maryland: US Army Laboratory Command, Ballistic Research Laboratory.

[2]Wheeler, CR. Toxicity testing of propellants. Laboratory Notebook #85-12-023.2, pp 17-23. Letterman Army Institute of Research, Presidio of San Francisco, CA.

ANALYSIS OF LIQUID PROPELLANT 1846 AND DOSING SOLUTIONS

LP1846 and the dosing solutions prepared from it were analyzed for percent hydroxylammonium nitrate (HAN) and triethanolammonium nitrate (TEAN) using an unpublished procedure supplied by Charles Leveritt.[1] In the first step (Step A) of the procedure, the propellant is titrated with a standardized alcoholic solution of potassium hydroxide (KOH). This titration yields the sum of the HAN and TEAN components. The second step (Step B) involves titration of the propellant after heating with benzaldehyde, providing the concentration of HAN alone. From the results of these two titrations the composition of the propellant is determined. Dosing solutions were analyzed by determining the concentration of HAN alone.

PROCEDURE:

An alcoholic solution of KOH was prepared by dissolving 12 g of reagent grade KOH and 200 mg of reagent grade barium nitrate in 12 ml of water. The solution was mixed, allowed to stand overnight, and filtered. The solution was then diluted to one liter with reagent-grade ethanol and the normality determined by titration with standard benzoic acid using phenolphthalein as the indicator. A second standardization with potassium hydrogen phthalate was also performed.

In Step A, each sample (denoted A_n) was added to a 150 ml flask and diluted with 50 ml of 95% ethanol. Two drops of phenolphthalein were added to each flask and the samples titrated to the first pink color lasting 30 seconds or more. In Step B, another set of samples (denoted B_n) were added to flasks containing 1 ml of benzaldehyde and 50 ml of ethanol. These samples were heated to 50 to 60°C for about 5 minutes and titrated using bromphenol blue as the indicator.

CALCULATIONS:

From Step B the percent HAN was determined as follows:

meq HAN in B_n = (ml KOH to titrate B_n) x (normality KOH soln)

% HAN = (meq HAN in B_n) x (96.04 mg HAN/1 meq HAN) x (1/weight B_n) x 100

Once the % HAN has been determined, the % TEAN and % water can be calculated from Step A:

meq HAN in A_n = (% HAN) x (weight A_n) x (1 meq HAN/96.04 mg HAN)

total meq (HAN + TEAN) = (ml KOH to titrate A_n) x (normality KOH soln)

meq TEAN = (total meq (HAN + TEAN)) - (meq HAN in A_n)

% TEAN = (meq TEAN) x (122.08 mg TEAN/1 meq TEAN) x (1/weight sample A_n) x 100

% WATER = 100 - (% HAN + % TEAN)

For the analysis of dosing solutions, the determination of the concentration of HAN was the only calculation required:

mg HAN/ml = (meq HAN) x (96.04 mg HAN/1 meq HAN)/(1 ml)

RESULTS:

LP1846 was analyzed on 16 July 86 using 0.1533 N KOH.[2] Sample weights and titrant volumes for Steps A and B are presented in Table 1.

TABLE 1. Analysis of LP1846.

	STEP A		STEP B	
	Sample Wt (mg)	KOH (ml)	Sample Wt (mg)	KOH (ml)
	248.7	12.91	242.1	10.11
	244.7	12.85	262.4	11.00
	270.8	13.65	246.5	10.29
			260.6	10.86
Average:	254.7	13.14	252.9	10.57

APPENDIX A

Based on these results the composition was determined to be 61.5% HAN, 18.4% TEAN, and 20.1% water.

The dosing solutions were analyzed by titration on 22 July 86 using 0.1574 N KOH.[3] Presented in Table 2 are the data obtained for Steps A and B of the analysis. The 50 mg HAN/ml and the 100 mg HAN/ml dosing solutions were determined to have actual concentrations of 54.6 mg HAN/ml and 106.0 mg HAN/ml, respectively.

TABLE 2. Analysis of Dosing Solutions.

TARGET CONCENTRATION (mg/ml)*	KOH (ml) STEP A	STEP B
50_1	4.48	3.64
50_2	4.21	3.59
100_1	8.17	7.04
100_2	8.31	6.99

*Two 1 ml aliquots of each dosing solution were analyzed.

[1]Leveritt CS. [Letter]. SUBJECT: Analysis of liquid propellants by titration (15 April 1986). Aberdeen Proving Ground, Maryland: US Army Laboratory Command, Ballistic Research Laboratory.

[2]Wheeler CR. Toxicity Testing of Propellants. Laboratory Notebook #85-12-023.2, pp 17-22, Letterman Army Institute of Research, Presidio of San Francisco, CA.

[3]*Ibid*, pp 24-31.

ANIMAL DATA

Species: Rattus norvegicus

Strain: Sprague-Dawley

Source: Bantin & Kingman
Laboratory Animal Consultants
3421 Yale Way
Fremont, CA 94538

Sex: Male

Age at dosing: 11 to 15 weeks

Condition of animals at start of study: Normal

Body weight range at dosing: 227-380 grams

Identification procedures: Ear tag, tag numbers inclusive: 204, 208, 227, 231, 233, 234, 236, 242, 248, 252, 271, 280-319, 357, 367, 370, 387, 393, 474-502

Pretest conditioning:

1. One week quarantine
2. Polyurethane catheters microsurgically implanted 3-5 days before dosing.
3. Food was removed the day prior to dosing

Justification: Previous studies on this compound have been performed on rats, and this species is a proven acceptable model for methemoglobin studies.

HISTORICAL LISTING OF STUDY EVENTS

Date	Event
10 Jun 86	Eleven animals were transferred from GLP 85021 to GLP 86004.
10 Jun-10 Oct 86	Animals were checked twice daily.
11 Jun 86	One animal was catheterized.
12 Jun 86	Two animals were catheterized.
13 Jun 86	Two animals were catheterized.
16 Jun 86	One animal was catheterized. One was sacrificed after catheter pulled out.
17 Jun 86	One animal was weighed and dosed with test compound. Blood pressure was monitored and blood samples acquired before and after dosing. Catheterized one animal. Weighed animals. Received shipment of 20 animals which were examined, eartagged, weighed, housed and fed.
18 Jun 86	One animal was catheterized. One animal was dosed. Follow-up blood samples were taken on previously dosed animal.
19 Jun 86	Three animals were catheterized.
20 Jun 86	One animal was dosed. Three animals were sacrificed.
23 Jun 86	One animal was dosed. Follow-up samples were taken on a previously dosed animal. Animals were weighed. Two animals were catheterized. Animals from 17 Jun shipment were removed from quarantine.
24 Jun 86	Two animals were catheterized. Two animals were dosed. Quality control animal (from 17 Jun shipment) was submitted to necropsy.
25 Jun 86	Two animals were catheterized. One animal was sacrificed. Received shipment of 20 animals which were examined, eartagged, weighed, housed and fed.
26 Jun 86	Three animals were catheterized. One animal was dosed and one animal was sacrificed. Quality control animal was submitted to necropsy from 25 Jun shipment.
27 Jun 86	Two animals were catheterized. Two animals were dosed. One animal was sacrificed. Follow-up blood samples were taken.

30 Jun 86	Sacrificed four animals. Weighed animals and took follow-up blood samples.
1 Jul 86	Dosed one animal. Sacrificed one animal.
2 Jul 86	Four animals were catheterized. Two animals were sacrificed.
3 Jul 86	Three animals were catheterized.
4 Jul 86	Animals from 17 Jun shipment were removed from quarantine.
7 Jul 86	Four animals were catheterized. Two animals were dosed. One animal was sacrificed.
8 Jul 86	Four animals were catheterized. Two animals were dosed. Follow-up blood samples were taken. Animals were weighed.
9 Jul 86	Follow-up blood samples were taken. One animal was sacrificed.
10 Jul 86	Four animals were catheterized. Two animals were dosed. One animal was sacrificed. Follow-up blood samples were taken.
11 Jul 86	One animal was dosed. Four animals were sacrificed. Follow-up blood samples were taken.
12 Jul 86	Follow-up blood samples were taken.
14 Jul 86	Three animals were catheterized. One animal was dosed. Follow-up blood samples were taken.
15 Jul 86	Animals were weighed. Six animals were sacrificed.
16 Jul 86	Follow-up blood samples were taken.
17 Jul 86	One animal was dosed. Two animals were sacrificed. Follow-up blood samples were taken.
18 Jul 86	Follow-up blood samples were taken.
21 Jul 86	Two animals were sacrificed. Follow-up blood samples were taken.
22 Jul 86	Animals were weighed.
25 Jul 86	Four animals were catheterized. Five animals were transferred to this study from N8601.
28 Jul 86	One animal was catheterized. One animal was dosed.
29 Jul 86	Animals were weighed. Two animals were catheterized. Three animals were sacrificed. Follow-up blood samples were taken.
30 Jul 86	Follow-up blood samples were taken.
31 Jul 86	Follow-up blood samples were taken.
1 Aug 86	Two animals were catheterized. One animal was dosed.

4 Aug 86	One animal was sacrificed. Follow-up blood samples were taken.
5 Aug 86	Animals were weighed. One animal was dosed.
6 Aug 86	One animal was dosed. Follow-up blood samples were taken.
7 Aug 86	Three animals were sacrificed. Follow-up blood samples were taken.
8 Aug 86	One animal was sacrificed. Follow-up blood samples were taken.
11 Aug 86	Follow-up blood samples were taken.
12 Aug 86	Follow-up blood samples were taken. 29 animals were received from Bantin & Kingman. They were examined, housed, and fed.
13 Aug 86	Follow-up blood samples were taken. Animals from 12 Aug shipment were eartagged and weighed, and a quality control animal was submitted to necropsy.
15 Aug 86	Follow-up blood samples were taken.
18 Aug 86	One animal was sacrificed. Follow-up blood samples were taken. 28 animals were received from quarantine (from 12 Aug shipment).
19 Aug 86	Eight animals were catheterized. Animals were weighed.
20 Aug 86	Four animals were catheterized. One animal was sacrificed.
21 Aug 86	Four animals were catheterized.
22 Aug 86	Three animals were catheterized. Two animals were dosed. One animal was sacrificed.
23 Aug 86	Two animals were dosed. Follow-up blood samples were taken.
24 Aug 86	Follow-up blood samples were taken.
25 Aug 86	Two animals were catheterized. One animal was dosed. Follow-up blood samples were taken.
26 Aug 86	One animal was dosed. Animals were weighed. Follow-up blood samples were taken.
27 Aug 86	Two animals were dosed. Follow-up blood samples were taken.
28 Aug 86	Two animals were dosed. Follow-up blood samples were taken.
29 Aug 86	Two animals were dosed. Follow-up blood samples were taken.
30 Aug 86	Follow-up blood samples were taken.
31 Aug 86	Follow-up blood samples were taken.
1 Sep 86	Follow-up blood samples were taken.
2 Sep 86	Animals were weighed. Follow-up blood

	samples were taken.
3 Sep 86	Follow-up blood samples were taken.
4 Sep 86	Two animals were catheterized. Follow-up blood samples were taken.
5 Sep 86	Two animals were catheterized. Follow-up blood samples were taken.
6 Sep 86	Follow-up blood samples were taken.
7 Sep 86	Three animals were catheterized. Follow-up blood samples were taken.
8 Sep 86	One animal was dosed. Follow-up blood samples were taken.
9 Sep 86	One animal was dosed. Follow-up blood samples were taken.
10 Sep 86	Animals were weighed. Follow-up blood samples were taken.
11 Sep 86	Three animals were dosed. One animal was sacrificed.
12 Sep 86	Follow-up blood samples were taken.
13 Sep 86	Follow-up blood samples were taken.
14 Sep 86	Follow-up blood samples were taken.
15 Sep 86	An animal was dosed to compare MHb values from catheter and tail vein.
16 Sep 86	Follow-up blood samples.
17 Sep 86	Follow-up blood samples.
19 Sep 86	An animal was dosed to stain for Heinz body verification.
22 Sep 86	Sacrificed one animal.
23 Sep 86	Sacrificed two animals. Animals were weighed.
30 Sep 86	Animals were weighed.
7 Oct 86	Animals were weighed. Three animals were dosed so that they could be used to verify Heinz bodies.
8 Oct 86	Examined blood for Heinz bodies.
10 Oct 86	Remaining animals were sacrificed.

APPENDIX C

BLOOD PRESSURE DATA
(mmHg)
VEHICLE CONTROL[a]

Animal#	280	291	296	316	479	484	490	495	n	MEAN	SD
Baseline	128	100	134	122	119	118	116	119	8	118.37	9.89
5 min.	132	118	130	112	116	103	124	112	8	118.38	9.82
15 min.	148	113	136	111	114	114	120	128	8	123.00	13.28
30 min.	136	134	118	112	113	117	119	117	8	120.88	9.00
60 min.	144	132	107	66	113	114	148	126	8	118.75	25.91

50mg(HAN)/kg[a]

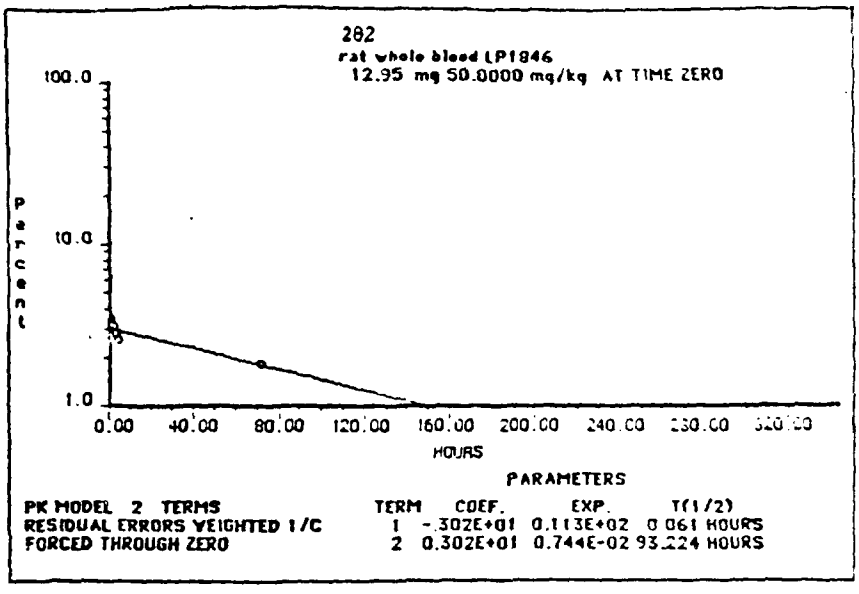
Animal#	282	293	300	308	474	481	487	493			
Baseline	144	130	100	118	100	109	125	135	8	120.13	16.26
5 min.	136	126	112	118	106	100	119	122	8	117.38	11.38
15 min.	144	124	117	123	102	104	118	136	8	121.00	14.35
30 min.	160	135	117	122	104	95	108	84	8	115.63	23.92
60 min.	160	120	123	121	103	100	116	140	8	122.88	19.45

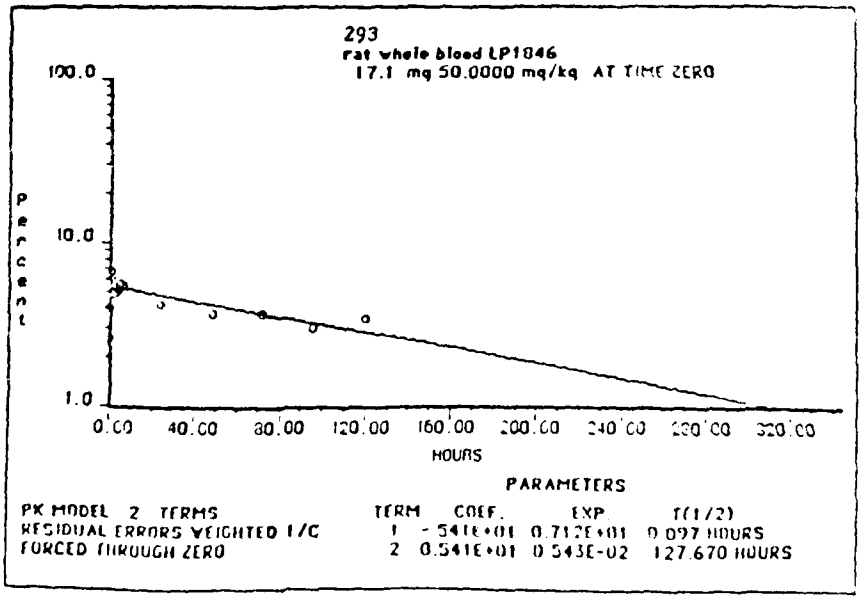
100 mg(HAN)/kg[a]

Animal #	297	301	314	387	482	486	489	494			
Baseline	144	134	115	130	118	108	128	112	8	123.63	12.35
5 min.	140	120	100	122	110	82	80	96	8	106.25	20.74
15 min.	144	126	134	141	120	94	90	106	8	119.38	20.76
30 min.	146	123	140	145	119	95	102	117	8	123.38	19.19
60 min.	152	110	145	146	114	92	94	126	8	122.38	23.64

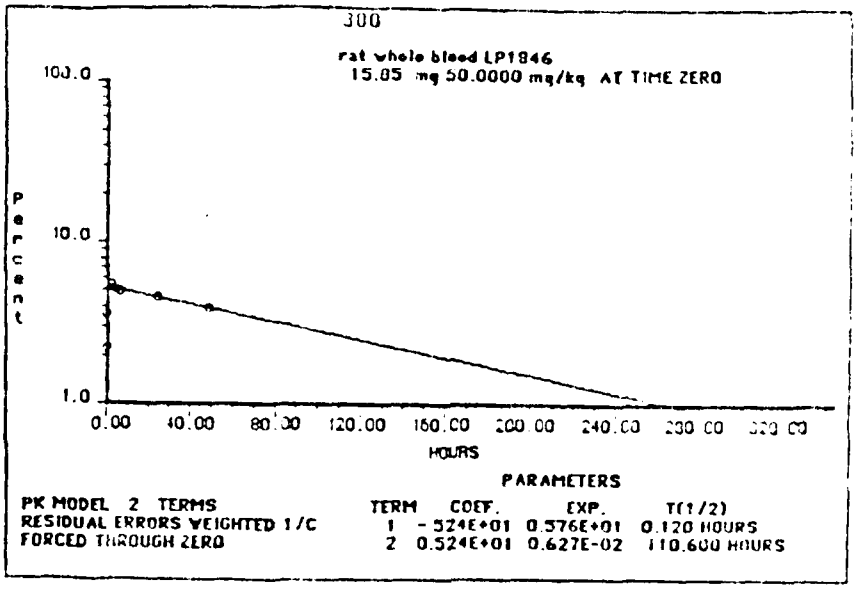
a. Abbreviations: n - Number of animals.
SD - Standard Deviation

METHEMOGLOBIN TIME VS CONCENTRATION CURVES





APPENDIX E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120.00
160.00
200.00
240.00
280.00
320.00
HOURS
PARAMETERS
PK MODEL 2 TERMS
RESIDUAL ERRORS WEIGHTED 1/C
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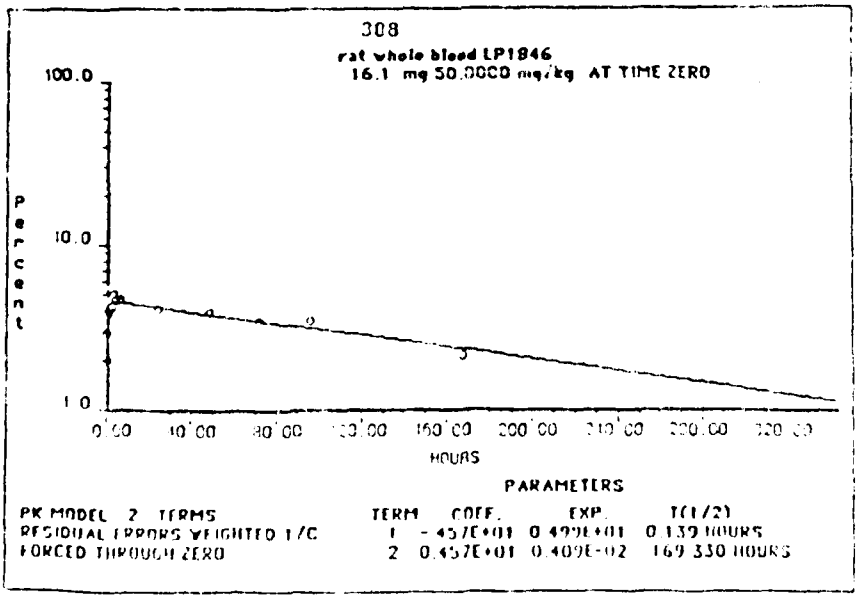
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160.00
200.00
240.00
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320.00
HOURS
PARAMETERS
PK MODEL 2 TERMS
RESIDUAL ERRORS WEIGHTED 1/C
FORCED THROUGH ZERO
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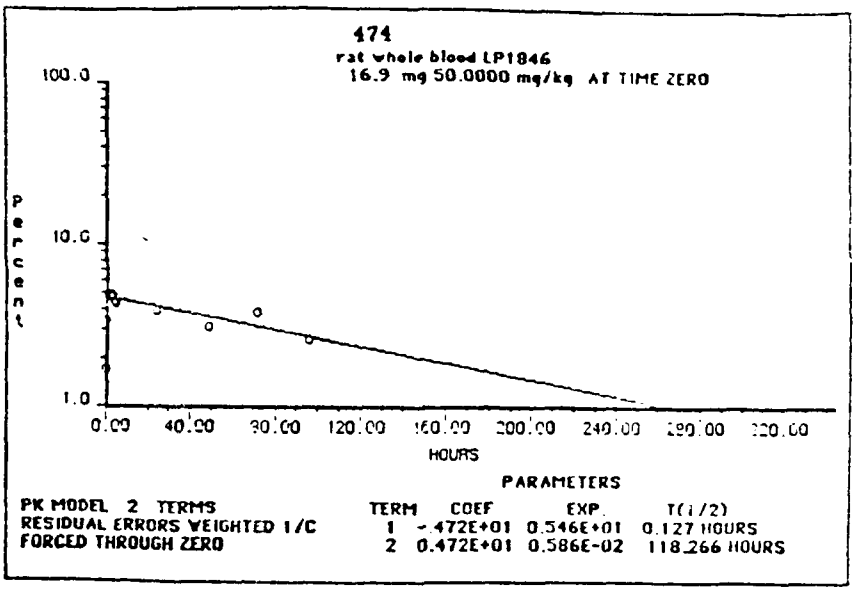
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PARAMETERS
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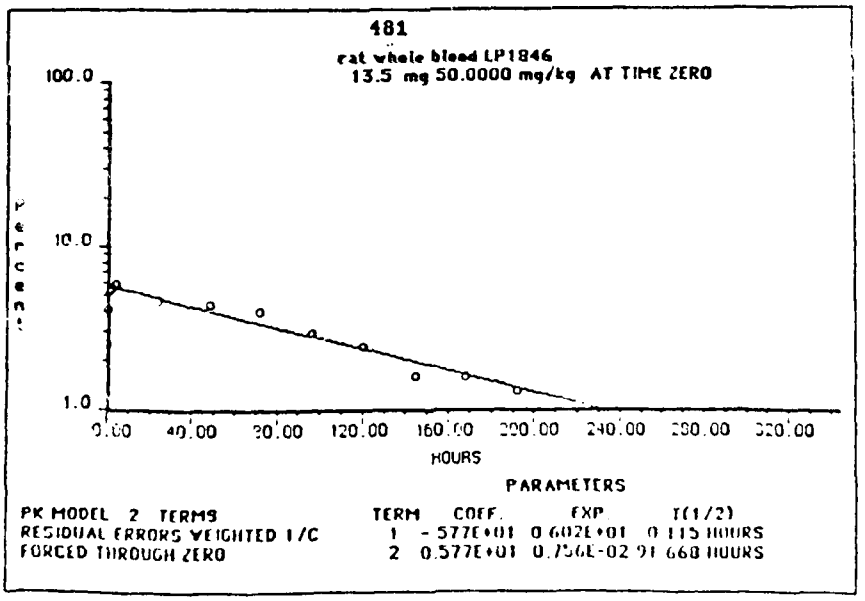
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120.00
160.00
200.00
240.00
280.00
320.00
HOURS
PARAMETERS
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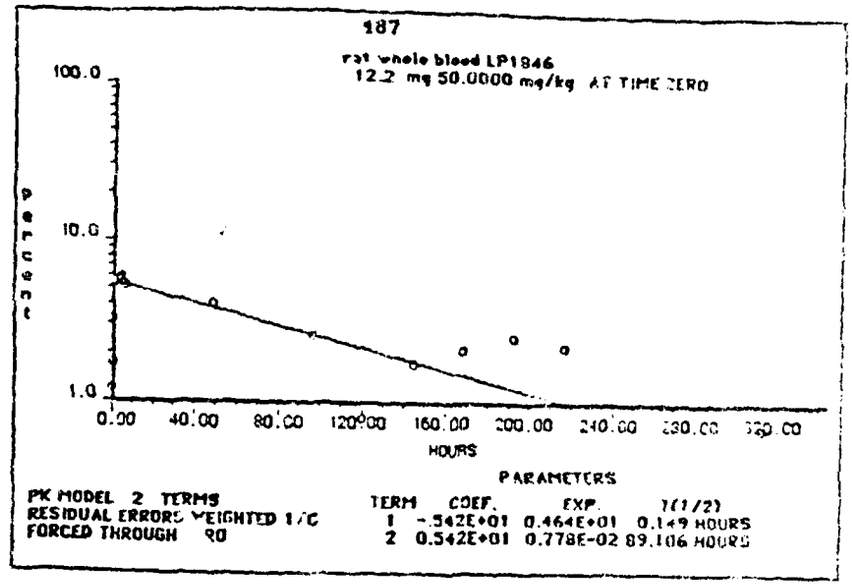
APPENDIX E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HOURS
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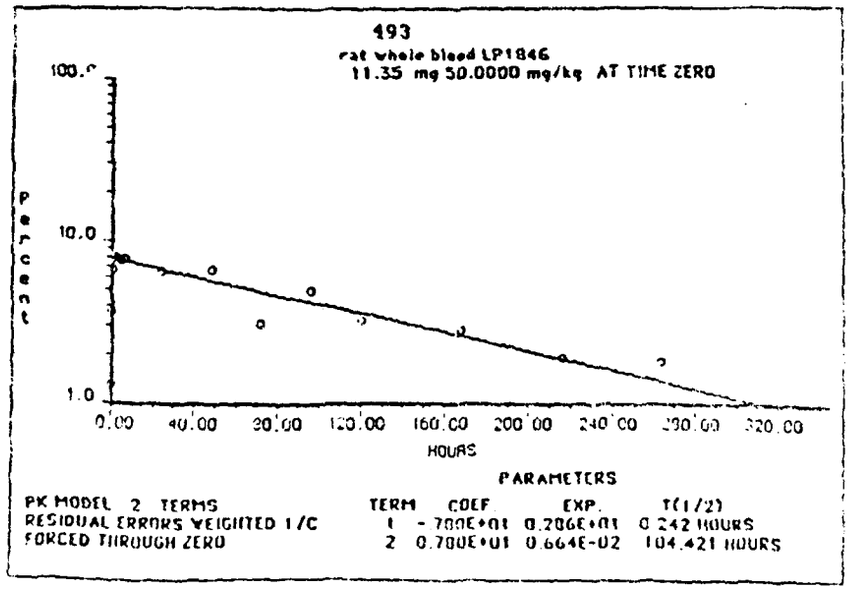
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HOURS
PARAMETERS
PK MODEL 2 TERMS
RESIDUAL ERRORS WEIGHTED 1/C
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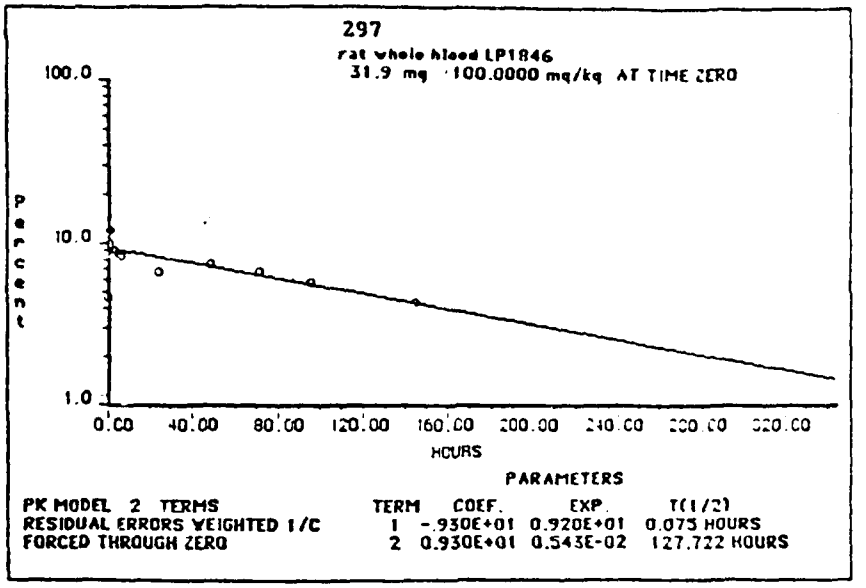
APPENDIX E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80.00
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TERM COEF. EXP. T(1/2)
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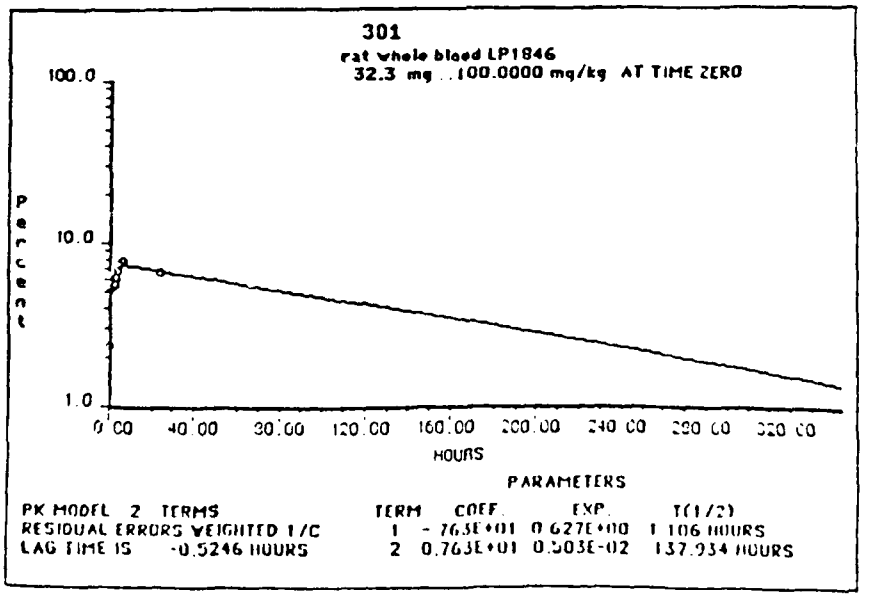
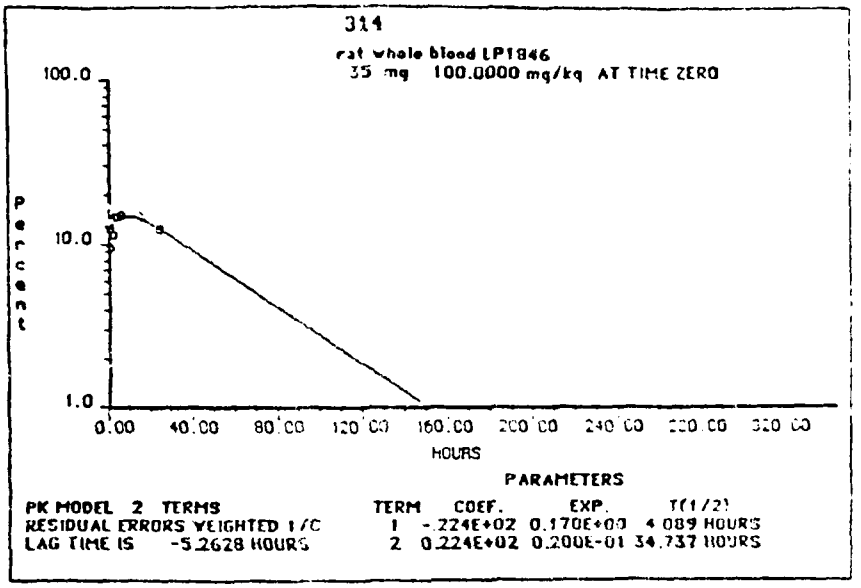
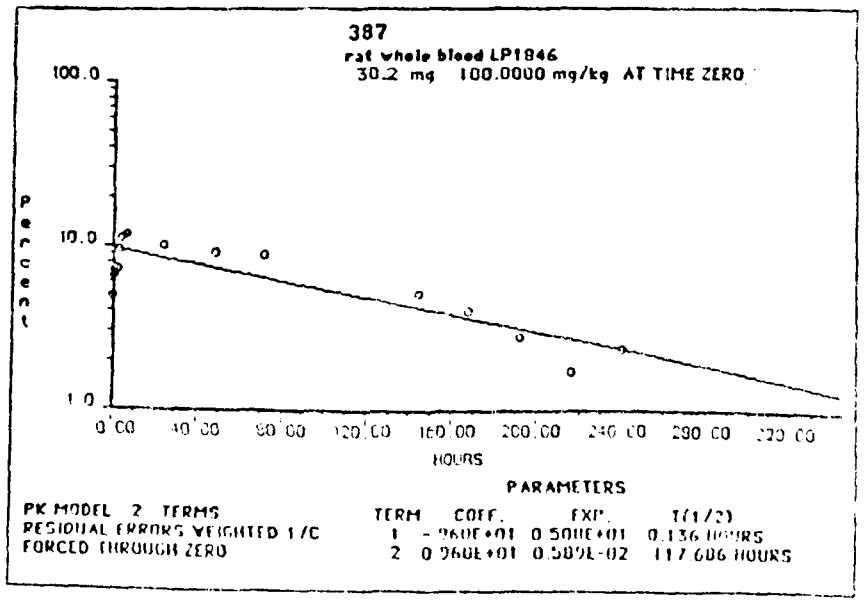
2 0.930E+01 0.543E-02 127.722 HOURS

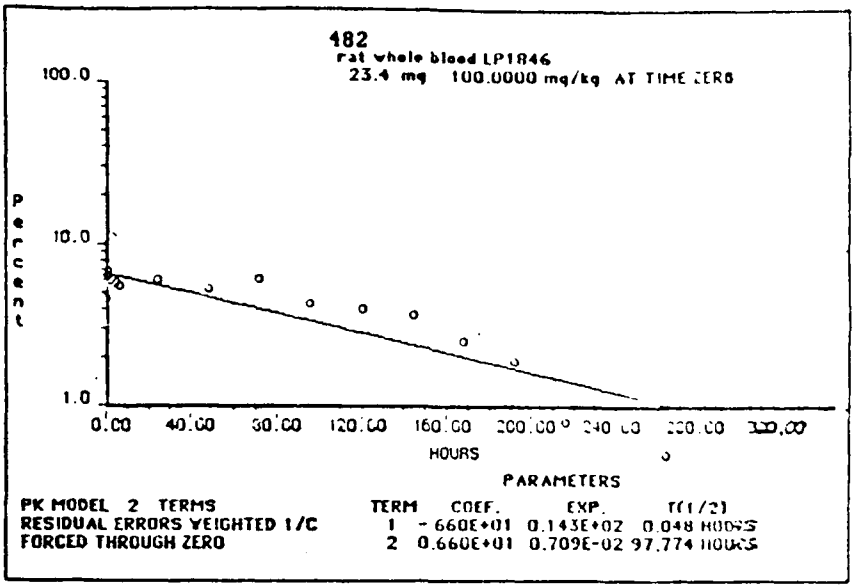

301
rat whole blood LP1846
32.3 mg 100.0000 mg/kg AT TIME ZERO
100.0
10.0
1.0
Percent
0.00
40.00
80.00
120.00
160.00
200.00
240.00
280.00
320.00
HOURS
PARAMETERS
PK MODEL 2 TERMS
RESIDUAL ERRORS WEIGHTED 1/C
LAG TIME IS -0.5246 HOURS
TERM COEF. EXP. T(1/2)
1 -.763E+01 0.627E+00 1.106 HOURS
2 0.763E+01 0.503E-02 137.934 HOURS

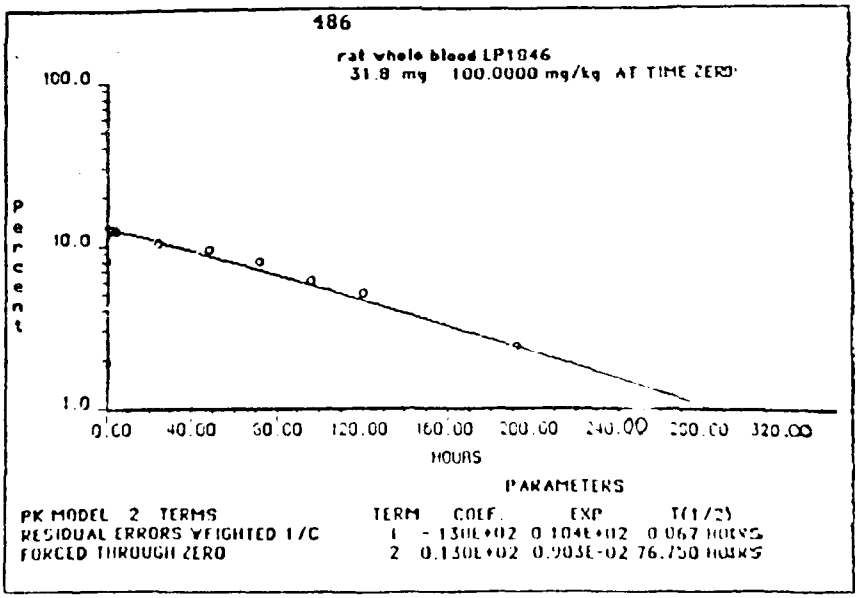
APPENDIX E


314
rat whole blood LP1846
35 mg 100.0000 mg/kg AT TIME ZERO
100.0
10.0
1.0
Percent
0.00
40.00
80.00
120.00
160.00
200.00
240.00
280.00
320.00
HOURS
PARAMETERS
PK MODEL 2 TERMS
RESIDUAL ERRORS WEIGHTED 1/C
LAG TIME IS -5.2628 HOURS
TERM COEF. EXP. T(1/2)
1 -.224E+02 0.170E+00 4.089 HOURS
2 0.224E+02 0.200E-01 34.737 HOURS

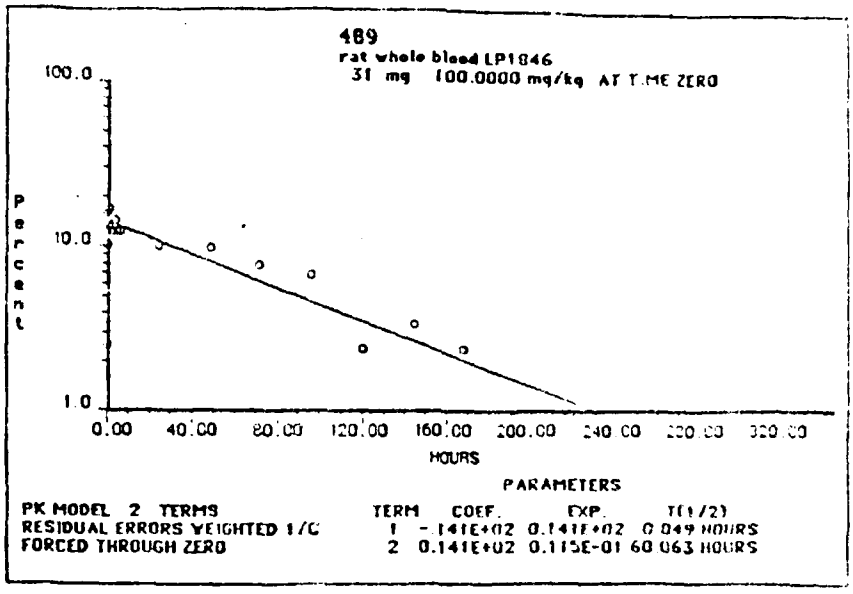

387
rat whole blood LP1846
30.2 mg 100.0000 mg/kg AT TIME ZERO
100.0
10.0
1.0
Percent
0.00
40.00
80.00
120.00
160.00
200.00
240.00
280.00
320.00
HOURS
PARAMETERS
PK MODEL 2 TERMS
RESIDUAL ERRORS WEIGHTED 1/C
FORCED THROUGH ZERO
TERM COEF. EXP. T(1/2)
1 -.960E+01 0.500E+01 0.136 HOURS
2 0.960E+01 0.589E-02 117.606 HOURS

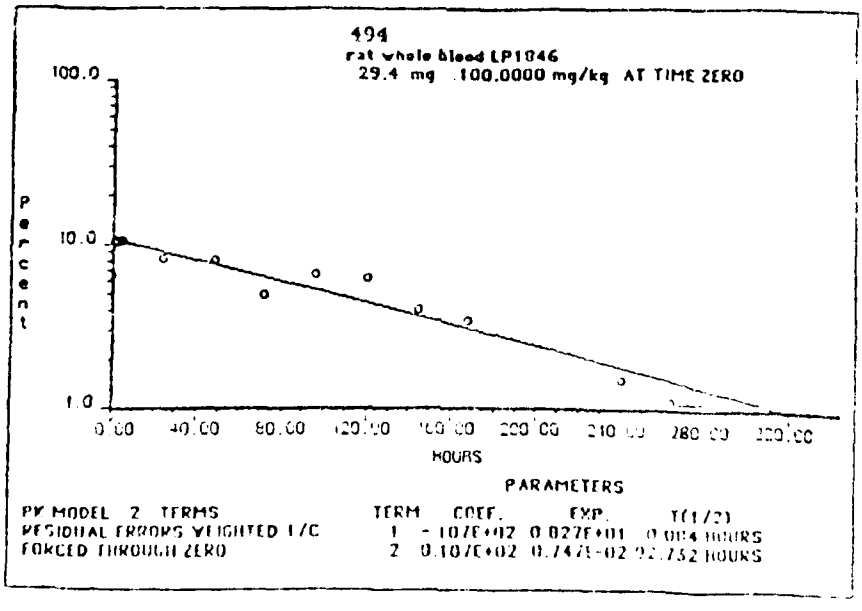
APPENDIX E


482
rat whole blood LP1846
23.4 mg 100.0000 mg/kg AT TIME ZERO
100.0
10.0
1.0
Percent
0.00
40.00
80.00
120.00
160.00
200.00
240.00
280.00
320.00
HOURS
PARAMETERS
PK MODEL 2 TERMS
RESIDUAL ERRORS WEIGHTED 1/C
FORCED THROUGH ZERO
TERM COEF. EXP. T(1/2)
1 -.660E+01 0.143E+02 0.048 HOURS
2 0.660E+01 0.709E-02 97.774 HOURS


486
rat whole blood LP1846
31.8 mg 100.0000 mg/kg AT TIME ZERO
100.0
10.0
1.0
Percent
0.00
40.00
80.00
120.00
160.00
200.00
240.00
280.00
320.00
HOURS
PARAMETERS
PK MODEL 2 TERMS
RESIDUAL ERRORS WEIGHTED 1/C
FORCED THROUGH ZERO
TERM COEF. EXP T(1/2)
1 -.150E+02 0.104E+02 0.067 HOURS
2 0.150E+02 0.903E-02 76.750 HOURS

APPENDIX E


489
rat whole blood LP1846
31 mg 100.0000 mg/kg AT TIME ZERO
100.0
10.0
1.0
Percent
0.00
40.00
80.00
120.00
160.00
200.00
240.00
280.00
320.00
HOURS
PARAMETERS
PK MODEL 2 TERMS
RESIDUAL ERRORS WEIGHTED 1/C
FORCED THROUGH ZERO
TERM COEF. EXP. T(1/2)
1 -.141E+02 0.141E+02 0.049 HOURS
2 0.141E+02 0.115E-01 60.063 HOURS


494
rat whole blood LP1846
29.4 mg 100.0000 mg/kg AT TIME ZERO
100.0
10.0
1.0
Percent
HOURS
PARAMETERS
PK MODEL 2 TERMS
RESIDUAL ERRORS WEIGHTED 1/C
FORCED THROUGH ZERO
TERM COEF. EXP. T(1/2)
1 -.107E+02 0.827E+01 0.084 HOURS
2 0.107E+02 0.747E-02 92.732 HOURS

APPENDIX E

METHEMOGLOBIN DATA[a]
VEHICLE CONTROL
(%)

Animal number	280	291	296	316	393	479	484	490	495	Mean	SD
Predose	1.0	1.2	1.2	1.5	1.0	0.9	1.3	0.9	1.1	1.12	0.20
1 min								0.9	0.9	0.90	0.00
5 min	1.1	1.6	1.0	1.0	1.0	1.2	1.0	1.2	0.9	1.11	0.21
15 min	1.4	1.0	1.3	1.0	1.1	1.2		1.1	1.2	1.16	0.14
30 min	1.1	1.3	1.0	0.8		0.9		0.9	1.4	1.06	0.22
60 min	1.1	1.2	0.6	1.0		1.2	1.4	1.3	0.9	1.09	0.25
120 min	1.9	1.1		1.3		0.9	1.1		1.1	1.23	0.35
180 min	1.0	1.4	1.0	0.8	0.8	1.2	1.8		1.0	1.13	0.34
240 min	1.1	1.5	1.0	1.3	1.4	1.1	1.2		1.3	1.24	0.17
300 min	1.0	1.0	1.5	0.8	1.2		1.1		0.9	1.07	0.23
360 min	1.0	1.4	1.5	1.3	1.1		1.2		1.5	1.29	0.20
24 hours	1.5	1.1	1.2	1.0	2.0	1.0	1.5	0.8	0.4	1.17	0.46
48 hours				1.1		1.2	1.2	0.4	1.0	0.98	0.33
72 hours				1.3		1.4	1.7		1.0	1.35	0.29
96 hours	0.9			1.3		1.7	0.2		1.0	1.02	0.55
120 hours						0.6	1.7		1.3	1.20	0.56
144 hours						1.3	0.4	1.5	1.2	1.10	0.48
168 hours				1.1		1.2	1.4	0.4	0.7	0.96	0.40
192 hours						0.9	0.8	0.5	0.4	0.65	0.24
216 hours							0.3			0.30	
240 hours							0.4			0.40	
264 hours						0.8				0.80	
288 hours						2.0				2.00	
312 hours						1.4				1.40	

a. The absence of data was the result of equipment failure (catheter or co-oximeter).

METHEMOGLOBIN DATA[a]
50 mg(HAN)/kg
(%)

Animal number	282	293	300	308	474	481	487	493	Mean	SD
Predose	1.0	1.0	0.9	1.0	1.3	1.4	1.1	1.3	1.13	0.18
1 min							1.2	1.3	1.25	0.07
5 min	3.2	2.6	2.2	2.0	1.7	2.5	1.7	1.3	2.15	0.61
15 min	2.8	4.0	3.6	2.9	3.4	4.1	3.2	3.7	3.46	0.48
30 min	3.4	6.0	5.2	3.9	4.8	5.6	5.2	6.6	5.09	1.05
60 min	3.2	6.6	5.2	4.1	4.9	5.3	5.4	7.7	5.30	1.39
120 min	3.1	4.9	5.4	4.2	4.9	5.5	5.5	7.9	5.18	1.37
180 min	2.8	5.3	5.2	5.0	4.8		5.7	7.8	5.23	1.47
240 min	2.8	5.0	5.0	4.6	4.4	5.8	5.9	7.6	5.14	1.39
300 min	2.6	5.5	5.0	4.7	4.3		5.4	7.5	5.00	1.47
360 min	3.5	5.4	4.9	4.7			5.2	7.8	5.25	1.42
24 hours		4.2	4.5	4.1	3.9	4.7		6.5	4.65	0.95
48 hours		3.7	3.9	3.9	3.1	4.4	4.0	6.7	4.24	1.15
72 hours	1.8	3.7		3.4	3.8	4.0		3.1	3.30	0.80
96 hours		3.0		3.4	2.6	2.9	2.6	4.9	3.23	0.87
120 hours		3.4				2.4	0.8	3.2	2.45	1.18
144 hours						1.6	1.7		1.65	0.07
168 hours				2.1		1.6	2.1	2.8	2.15	0.49
192 hours						1.3	2.5		1.90	0.85
216 hours						0.2	2.2	1.9	1.43	1.08
240 hours						0.5			0.50	
264 hours							1.0	1.8	1.40	0.57
288 hours										
312 hours						1.7	0.7		1.20	0.70

a. The absence of data was the result of equipment failure (catheter or co-oximeter).

METHEMOGLOBIN DATA[a]
100 mg(HAN)/kg
(%)

Animal Number	297	301	314	387	482	486	489	494	Mean	SD
Predose	1.2	1.1	1.3	1.1	1.2	1.2	1.2		1.19	0.07
1 min						1.9	2.5	0.9	1.77	0.81
5 min	4.6	2.3	11.4	4.9	4.6	8.1	10.2	6.5	6.58	3.10
15 min	9.1		13.0	6.4	6.4	11.3	15.9	9.4	10.21	3.47
30 min	11.9		12.5	7.1	6.9	13.0	16.5	10.3	11.17	3.41
60 min	10.1	5.3	9.6	6.6	6.3	12.8	12.9	10.7	9.29	2.93
120 min	9.0	5.6	11.4	7.1	6.0	12.5	12.2	10.6	9.30	2.78
180 min	9.0	6.2	14.8	9.5	6.1	12.4	12.2	10.2	10.05	3.04
240 min	8.8	6.9	14.6	11.2	5.7	12.5	14.4	10.5	10.58	3.28
300 min	8.6	7.3	14.6	11.4	5.9	12.3	12.3	10.4	10.35	2.90
360 min	8.3	7.7	14.9	11.7	5.5		12.4		10.08	3.49
24 hours	6.6	6.7	12.2	10.0	6.0	10.2	9.9	8.1	8.71	2.19
48 hours	7.4			9.0	5.3	9.3	9.7	8.0	8.12	1.62
72 hours	6.6			8.7	6.2	7.8	7.8	4.9	7.00	1.37
96 hours	5.7				4.3	5.9	6.8	6.4	5.82	0.95
120 hours					4.0	4.9	2.4	6.1	4.35	1.56
144 hours	4.3			5.0	3.7		3.4	4.0	4.08	0.61
168 hours				4.0	2.5		2.4	3.4	3.08	0.76
192 hours				2.8	1.9	2.3	1.0		2.00	0.76
216 hours				1.7	0.8		1.0		1.17	0.47
240 hours				2.3		1.0		1.5	1.61	0.66
264 hours					0.5			1.1	0.81	0.42

a. The absence of data was the result of equipment failure (catheter or co-oximeter).

APPENDIX F

METHEMOGLOBIN KINETICS

50 mg (HAN)/kg

	Animal number	T 1/2 (hrs)	AUC (% * hrs)	T-Max (hrs)	C-Max (%)
	282	93.224	406.095	0.646	3.0
	293	127.670	995.321	1.008	5.4
	300	110.600	834.601	1.187	5.2
	308	169.330	1114.928	1.426	4.5
	474	118.266	805.269	1.253	4.7
	481	91.668	761.459	1.111	5.7
	487	89.106	695.455	1.380	5.4
	493	104.421	1172.944	2.123	7.7
Mean		113.036	848.259	1.267	5.2
SD		26.491	246.862	0.423	1.3
SEM		9.366	87.279	0.150	0.5

100 mg (HAN)/kg

	Animal number	T 1/2 (hrs)	AUC (% * hrs)	T-Max (hrs)	C-Max (%)
	297	127.722	1713.381	0.808	9.3
	301	137.934	1505.523	7.239	7.3
	314	34.737	990.774	9.042	14.9
	387	117.686	1628.541	1.331	9.5
	482	97.774	931.178	0.531	6.6
	486	76.750	1443.706	0.681	13.0
	489	60.063	1220.285	0.505	14.0
	494	92.732	1430.515	0.848	10.6
Mean		93.175	1357.988	2.623	10.6
SD		35.051	285.320	3.449	3.1
SEM		12.392	100.876	1.219	1.1

AUC = area under the time-concentration curve
T-Max = time to peak concentration
C-Max = peak concentration
T 1/2 = half-time of methemoglobin reduction

HEINZ BODY DATA

Time (after dosing) of First Appearance

50 mg(HAN)/kg		100 mg(HAN)/kg	
ANIMAL NUMBER	TIME (hrs)	ANIMAL NUMBER	TIME (hrs)
282	2	297	3
293	48	301	2
300	4	314	2
308	6	387	2
474	5	482	4
481	4	486	1
487	5	489	5
493	1	494	1

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